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DNA-microarrays and food-biotechnology

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The recent boost in bacterial genome sequences (e.g. Kunst et al. 1997; Bolotin et al. 1999) now enables true 'functional genomics' studies, including transcriptome- proteome- and metabolome analyses as well as structural genomics. For these areas novel high-throughput analytical methods have been developed, such as DNA-microarrays and improved 2D-electrophoresis methods combined with MALDI-TOF analyses. The large number of experimental data generated are gathered into large data-bases, the interpretation of which will greatly depend on novel bioinformatics methodologies, which should enable linking of the databases and facilitate classification and interpretation of the results.

DNA-microarrays provide a powerful tool for analyzing transcription profiles of whole genomes of any organism (Schena et al. 1998, Graves 1999). An effective way of producing DNA-microarrays is to spot amplicons of each ORF annotated in the genome sequence of interest on a defined support material. Another type of DNA-chip is provided by Affymetrix, a company that synthesizes oligonucleotides on solid materials by photolithography (for example De Saizieu et al. 1998). Bacterial genomes of Gram-positive bacteria typically harbour between 2000 and 5000 genes. Amplicons can conveniently be generated by PCR, and subsequently spotted onto membrane filters or glass-slides. On glass-slides over 100.000 spots per cm^2 can be accommodated, providing sufficient combinatorial possibilities for bacterial genome applications. Total RNA is isolated from the wild-type strain and from the isogenic strain under investigation, e.g. a strain containing a specific mutation or grown under different conditions. Subsequently, cDNA is made while incorporating either α-^{33}P-dCTP label (for membrane filters) or Cy3/Cy5 fluorescent label (for glass-slides). The labeled cDNA is then used for hybridization to the DNA-arrays and the signals are detected by phosphor-imaging (filters) or confocal laser scanning (glass-slides). In the latter case the wild-type and mutant-strain cDNA's are differentially labelled and these can be used in one combined sample for hybridization, providing the attractive possibility of multiplexing. The data obtained provide information on differential gene expression, which is extremely useful for studying pleiotropic regulatory effects, e.g. those involved in stress response, in complex regulatory cascades and in resistance mechanisms against antibiotics, antimicrobial peptides, phages and/or stress conditions.

We recently applied the DNA-array screening technology as a new and powerful tool to study gene regulation in *Bacillus subtilis*. This Gram-positive soil bacterium has developed a wide variety of adaptations to cope with the strong nutritional and physico-chemical fluctuations which occur in its environment. A motility system in concert with a chemotactic sensing device offers the bacterium the possibility to search for better growth and/or survival conditions. When nutrient limitations continue, *B. subtilis* cells start to secrete degradative enzymes and antibiotics, and acquire the ability to differentiate into cells competent for genetic transformation. Prolonged nutritional deprivation ultimately results in the production of dormant spores. *B. subtilis* employs elaborate systems for sensing the optimal conditions to initiate and regulate these different adaptations. The regulation of competence development has been studied in our laboratory for many years. Competence development is initiated when exponential growth ceases, and is optimal in minimal medium with glucose as the sole carbon source. A sufficiently high cell density is another prerequisite for optimal competence. The various environmental signals are interpreted by a complex signal transduction pathway and ultimately result in the synthesis of the competence transcription factor, encoded by *comK*.

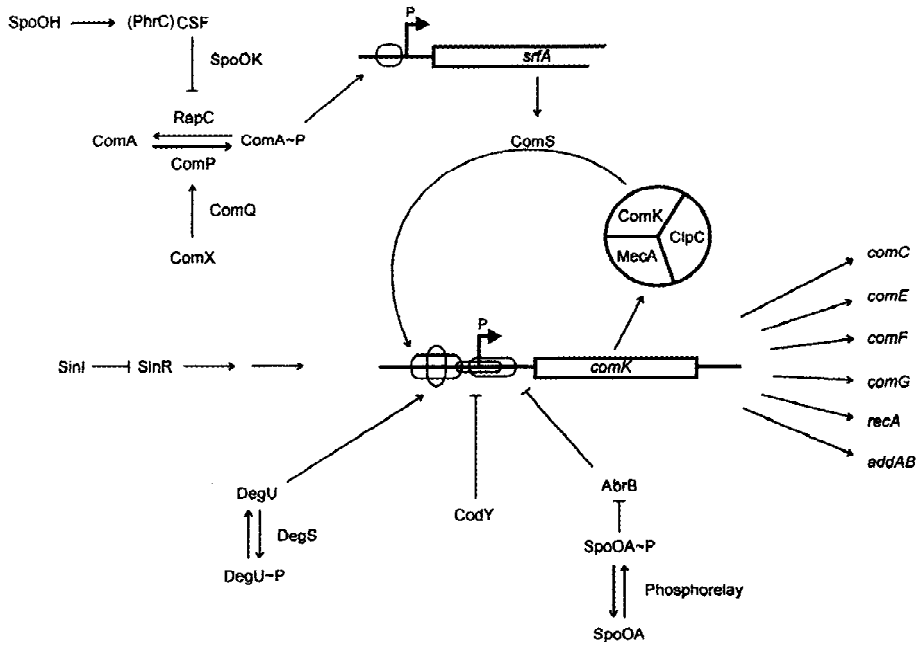

Figure 1. Regulation via the competence signal transduction cascade ultimately results in the synthesis of the competence transcription factor, ComK, which activates the transcription of the late competence genes encoding the DNA binding and uptake machinery (*comC, comE, comF, comG*), as well as of genes necessary for recombination (*recA, addAB*). During exponential growth, ComK is kept inactive by the formation of a ternary complex with MecA and the chaperone-like protein ClpC. Towards the end of the exponential growth phase, two quorum sensing pheromones, ComX and CSF, accumulate in the medium, activating a pathway which results in the phosphorylation of the response regulator ComA. ComA P activates the transcription of *srfA*, which encodes the synthetase of the lipopeptide antibiotic surfactin. Embedded within the *srfA* operon is a small gene,*comS*. The synthesis of ComS in response to the quorum-sensing signal transduction pathway destabilizes the ternary ComK/MecA/ClpC complex in which ComK is held inactive. Since ComK synthesis is positively autoregulated, the release of active ComK from the ternary complex results in the rapid accumulation of ComK. In addition to ComK itself, the DNA binding proteins DegU, AbrB and SinR are also required for the transcriptional activation of *comK*. For full competence development repression at the *comK* promoter due to the binding of AbrB and CodY must be relieved as well.

ComK activates the transcription of the late competence genes, encoding the DNA binding and uptake machinery (*comC*, *comE*, *comF*, *comG*), as well as transcription of genes necessary for DNA recombination, such as *recA* and *addAB*. In addition, ComK is also required for the expression of its own gene. The ComK-binding motif has been identified (Hamoen et al. 1998), and the signal transduction cascade leading to the expression of ComK has been elucidated in great detail (Figure 1).

However, several important questions remain and genome-wide expression profiling experiments are currently being used to obtain answers to these questions. To fully understand the mechanism of competence it is necessary to identify the proteins of the DNA uptake and integration systems which are activated during competence. As such genes are regulated by ComK, we searched for ComK activated genes. Several of these genes have been identified (Figure 1). When the entire genome sequence of *B. subtilis* was screened for possible functional ComK binding sites, more than hundred potential ComK-activated genes were found. DNA-microarrays comprising all open reading frames of *B. subtilis* were used to determine additional ComK-regulated genes. By comparing the genome-wide expression profiles of a wild type *B. subtilis* strain with a strain in which the *comK* gene is deleted, we are gaining insight in the gene products likely to be required for the DNA binding, translocation, and recombination processes that make *B. subtilis* cells competent for genetic transformation.

Of course, specific DNA-arrays can be developed for various other purposes, e.g. rapid identification of pathogens and spoilage bacteria, mutation analyses, and for studying DNA-protein interactions. Morever, also RNA of related or even less-related species can be used for differential gene expression, using less stringent hybridization conditions, providing for virtual

expression arrays (VEA, Garner 1999). The combination of DNA-microarrays with proteome research and the elucidation of structure/function relationships of biomolecules will eventually result in a true understanding of whole cell functioning. On the short term it will stimulate various areas of microbiological, genetic and biochemical research, directed towards a better understanding of metabolic pathways, complex regulatory mechanisms and novel bioactivities, forming an indispensable knowledge-base in modern biotechnology. Finally, it is expected that many more genome sequences of lactic acid bacteria will become available in the near future, including those of several *Lactobacillus* species and that of *Streptococcus thermophilus*, which creates attractive possibilities for studying the latter organisms in their industrial environments or in their host. On the side of pathogens and food spoilage bacteria a fast increase in sequencing data is also taking place, with organisms as *Listeria monocytogenes*, *Bacillus* sp. and *Clostridium* sp. being sequenced at the moment, creating exciting possibilities for the development of novel antimicrobial strategies, and the study of their *in situ* activities.

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